In vitro Evaluation of Skin Distribution and Human Dermal Papilla Cells Proliferation for Minoxidil 5% Topical Lotion Hydrogel

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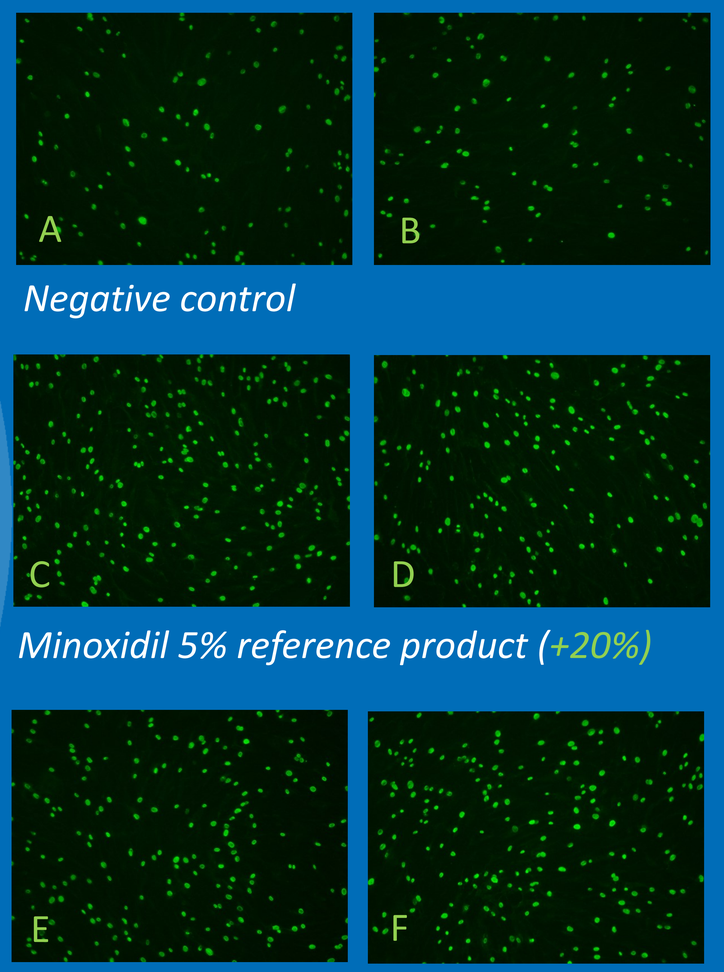
Introduction: Androgenetic alopecia (AGA) is the most common type of progressive hair loss, and it is estimated to affect 50 million men in the United States. Although it is neither life threatening nor painful, it is a distressful condition that may lead to psychosocial consequences[1]. For decades, the treatment of reference for AGA has been the topical application of minoxidil 2% or 5%, which is commercially available as a solution and a foam. When compounded, minoxidil may be incorporated in variable strengths to a topical base in order to meet the individual needs of men. An alternative topical compounded formulation was developed to include minoxidil in a proprietary hydrogel (Atrevis Hydrogel®) which is pharmaceutically elegant, alcohol-free to reduce the skin irritation, and it does not require pH adjustment to facilitate the pharmacy compounding process[2].

Materials and Methods: The bromodeoxyuridine (BrdU) staining was the assay used to evaluate *in vitro* the proliferation of human hair dermal papilla cells, which are located at the bottom of hair follicles. The cells were exposed to minoxidil 5% topical lotion hydrogel (compounded formulation), and to minoxidil 5% reference (commercial) product over a period of 24 hours. Untreated cells were used as negative control, whereas the commercial product of reference was used as positive control. The BrdU is an analog of the nucleoside thymidine commonly used to identify proliferating cells and, when incorporated into nuclear DNA, it represents a label that can be tracked using antibody probes to detect DNA synthesis[3]. Additional *in vitro* studies evaluated the percutaneous absorption (distribution) of minoxidil across the skin using the Franz Skin Finite Dose Model[4], following 3 hours of exposure.

Results and Discussion: For the proliferation analysis, the BrdU staining assay identified the proliferating human dermal papilla cells upon 24 hours of exposure by minoxidil 5% (commercial product versus compounded formulation), using fluorescence detection. The proliferation effect of minoxidil was significant in both minoxidil commercial (RFU 181,446 ±38,171, p=0.021) and minoxidil compounded (RFU 183,109±24,416, p=0.006), when compared to the negative control. As displayed in Figure 1 (A-F), the corresponding percentages of change were 22% for the compounded formulation versus 20% for the commercial product.

For the skin distribution, the Franz Skin Finite Dose Model evaluated the percutaneous absorption of minoxidil into the dermis and receptor medium using skin samples from two male Caucasian donors. Following a skin integrity test and 3 hours of diffusion, the minoxidil was extracted from the dermis layer and analysed by UPLC. It was shown that the percutaneous absorption into the dermis was similar for the minoxidil compounded (13.710 µg ± 4.660) and the minoxidil commercial (13.980 µg ± 3.220). Likewise, the percutaneous absorption into the receptor medium was also similar for both test and positive control (≈ 0 µg).



Negative control

Minoxidil 5% reference product (+20%)

Minoxidil 5% topical lotion hydrogel (+22%)

Figure 1 (A-F). Proliferation of human dermal papilla cells upon treatment with minoxidil 5% topical lotion hydrogel (E,F), and also minoxidil 5% reference product (C,D), in comparison to a negative control (A,B), over a period of 24 hours. Images were taken under fluorescent microscopy (Nikon, Japan) .

Conclusions: The *in vitro* performance of the minoxidil 5% topical lotion hydrogel was comparable to the commercial product of reference. As such, the easy to compound, alcohol-free formulation is a promising therapeutic alternative in AGA.

References:

1. Cash TF. The psychology of hair loss and its implications for patient care. *Clin Dermatol*. 2001;19(2):161-166.

2. PCCA. *Atrevis Hydrogel®* (30-4986). Available at: https://www.pccarx.com/products/PCCA-ATREVIS-HYDROGEL%C2%AE/30-4986/PROPRIETARYBASES (Last accessed: May 10th, 2022).

3. abcam (2021) *BrdU staining and BrdU assay protocol.* Available at: https://www.abcam.com/protocols/brdu-staining-protocol (Last accessed: May 10th, 2022).

4. Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. *Skin Pharmacol Phys* 2009;22:276–86.

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